PROTOCOL

STUDY TITLE

Performance Evaluation of Fresh Cab Mouse Repellant for Storage Areas on Wild House Mice (*Mus musculus*)

AUTHOR

Jeff J. Mach, Study Director

PERFORMING LABORATORY

Genesis Laboratories, Inc.
10122 Northeast Frontage Road
Wellington, CO 80549

STUDY NUMBER

N05007

SPONSOR

Crane Creek Gardens
17 3rd Ave, SE
Stanley, ND 58784

N05007 Genesis Laboratories, Inc. Page 2

PROTOCOL ACCEPTANCE

Test Device: Fresh Cab Mouse Repellant for Storage Areas

Study Number: N05007

Laboratory Performing All Experimentation In This Protocol:

Genesis Laboratories, Inc.
10122 Northeast Frontage Road
Wellington, Colorado 80549

Jeff J. Mach
Study Director
Genesis Laboratories, Inc.

4/21/05
Date

Richard Poché
Technical Manager
Genesis Laboratories, Inc.

4/21/05
Date

Kari J Warberg
Kari Warberg
Founder & CEO
Crane Creek Gardens

4-21-05
Date

TABLE OF CONTENTS

PURPOSE

The purpose of this study is to determine the level of repellency of Fresh Cab to wild house mice (*Mus musculus*). Fresh Cab is a package of various herbal ingredients that emit a scent, thought to be unattractive to house mice.

TEST DEVICE

The test substance, Fresh Cab, was developed by Crane Creek Gardens for repelling commensal rodents from farm machinery and vehicles. It emits a natural odor that fills the available space, as in a tractor cab. Its purpose is to rid the vehicles or storage areas from mice that often bed in the upholstery foam and paper materials and cause aesthetic damage, defecate on personal property, and gnaw on electrical cables.

PROPOSED EXPERIMENTAL START AND TERMINATION DATES

The proposed experimental start date for this study is April 1, 2005 and the experimental termination date will be approximately June 1, 2004. The actual dates will be specified in the final report.

TEST SYSTEM

Species - House mouse (*Mus musculus*)

Justification for Selection – House mice are commensal rodents that may frequent domestic domiciles and storage areas.

Size and Age – Each animal may be of any size or age.

Sex – Approximately equal numbers will be used for each sex.

Number – Five animals will be used for each replication of the test. Ten replicates are required. Up to 10 additional animals may be attained to assure healthy animals are used for each test. A total of 60 animals will be required.

Source – The animals will be wild-caught by Genesis Laboratories, Inc. from nearby farms or residences. Sherman live-traps will be used to capture the mice. The traps will also be baited with non-toxic oats and peanut butter for a moderate food supply, propylene batting will be added for bedding and protection from any colder temperatures. The mice will be maintained in the live-traps until they are released within Genesis Labs. Mice will be held in the cab of any transport vehicle to assure a suitable transport environment.

Housing and Husbandry - All animals will be logged in upon receipt according to the current version of SOP AS-1. The test animals will be inspected for general health as soon as possible after arrival at the testing facility. A test room will be reserved exclusively for the mice used in this study. Animals will be sexed and placed individually in temporary containment arenas. The arenas will be

oval-shaped stock tank with a volume of 20.56 ft^3 (sold as 2' x 4' x 2' tanks). Test animals shall be held under conditions designed to minimize disease potential (e.g. held in cages constructed from nonabsorbent materials, out of contact with excreta, and provided with clean feed and water). Bedding changes, and cleaning of watering apparatus, and cages will be done according to the current version of Genesis Laboratories, SOP AS-11.

Pre-test Holding - All candidate animals used in the study may be held for a holding period, but they may also be used immediately upon arrival to the facility. Temperature, humidity, and lighting will be provided as appropriate according to Guide for the Care and Use of Laboratory Animals (Regional Resource Council 1996).

FEED AND WATER FOR THE TEST SYSTEM

Pre-test - Fresh Purina Rodent Diet 5001 will be available *ad libitum* during the pre-test holding period. Water will be available *ad libitum*.

Test - Fresh Purina Rodent Diet 5001 will be available *ad libitum* during the testing period. Water will be available *ad libitum*.

EXPERIMENTAL DESIGN

Test Rooms - Two (2) tank enclosures will be used as the test and control areas for each replicate. Multiple replicates may be conducted at one time. Transparent plastic tubing or shaped hardware cloth tubes will connect the two enclosures with a "T" fitting in the center of the tubing where the mice will be added to the test apparatus. One enclosure will serve as the treatment area and one enclosure will serve as the control. Designation of treatment and control areas will be randomly determined prior to start of an evaluation. The Fresh Cab will be placed as the label designates. Food, water and harborage will be provided in each enclosure.

Environment - Temperature within the test room will be maintained at approximately 15-25°C. Strong air currents from heaters or air conditioners will not blow directly onto test animals. A photoperiod of 12 hours light: 12 hours dark shall be maintained for the duration of the test. During the 12-hour dark period, red light will be used in the test rooms to facilitate video surveillance. The relative humidity at the cage location is preferred to be at 30-70%. The test room will be kept clean.

EVALUATION OF THE TEST DEVICE

Exposure Period - Testing will begin by placing the Fresh Cab into one of the enclosures two hours prior to placement of the animals. The exposure period will last for 30 days.

Post-test Period - No post-test period will be initiated.

PARAMETERS TO BE INVESTIGATED

Feed Consumption – The amounts of Fresh Purina Rodent Diet 5001 consumed will be measured

approximately every 3 days. Spilled diet will be returned to the appropriate container before weighing. Consumption will aid in determining activity within the test and control environment. .

Observations – Test animals will be observed on a daily basis for health and their current position. The current position will be evaluated by counting the mice and recording in what container were they located.

Body Weights - Body weights will be recorded as the study animals are placed on test and at the termination of each replicate. and @ death.

PROPOSED STATISTICAL METHODS AND EVALUATION OF RESULTS

Statistic 1 - Amount of diet consumed in the treatment enclosure will be compared to diet consumed in the control enclosure during the exposure period by use of Chi-square test or other appropriate means.

To document repellency for the test device, it is proposed that diet consumption in the control enclosure will be much greater than diet consumption in the treatment enclosure (statistically significant difference $p < 0.05$) during the exposure period.

Statistic 2 - Observation counts will be used as a method for assessing activity. Observations will be collected at various times during the day as we observe the animals as to their health.

To document repellency for the test device, it is proposed that amount of time spent in the control enclosure will be much greater than time spent in the treatment enclosure (statistically significant difference $p < 0.05$) during the exposure period. The Chi-square test or other appropriate means will be used to analyze the data.

RECORDS TO BE MAINTAINED

All original data generated in support of this non-GLP study will be documented according to Genesis Laboratories Standard Operating Procedures. All data will be verified and maintained in folders in the raw data file. Other comments, descriptions, calculations, correspondence and other study related documents, will also be placed in the raw data file. The records will include but not limited to the following.

Protocol - A copy of the approved protocol and copies of all approved amendments will be maintained.

Standard Operating Procedures (SOP) - A list of all SOPs used during the conduct of the study and all SOP deviations will be maintained.

Personnel - A listing of all persons involved in the conduct, supervision, review or approval of any aspect of this study, including name, job title, authentic signature, (including signed initials). Current curriculum vitaes and training records will be held on file at Genesis Laboratories.

Test Substance - Records will be maintained of the identification of the test substance, all specifications obtained from the Sponsor or other sources, and copies of records of chain of custody and lot number.

Test Substance Utilization - The test substance in terms of concentration, batch homogeneity, and test substance accountability of all amounts used will be maintained.

Animals - Records will be maintained of the receipt of the test system used and original data regarding observations made during the test.

Correspondence and Telephone Logs - Records of all written and verbal communications critical to the conduct of the study.

Other - Records of all other original data generated during the course of study.

Archives – All original data, original protocol, final report, an electronic copy, and copies of logbooks necessary for a study will be archived at the sponsor company or their designee. Genesis Laboratories, Inc. will archive all data maintained in logbooks, and at a minimum, a hard copy of the protocol and final report.

PROTOCOL AMENDMENTS

All protocol amendments will be expressed in writing, and will be signed and dated by the Study Director. Amendments will usually be issued prior to initiation of the protocol change. However, when a change is required without sufficient time to issue a written amendment, the change may be communicated verbally by the Study Director to the Sponsor. The verbal notice will be followed with a written amendment as soon as possible. In this case, the effective date of the written amendment will be the date of the verbal change. This procedure is detailed in the current version of SOP SI-3. Copies of all signed amendments will be appended to the final report.

PROTOCOL DEVIATIONS

Any deviation from the protocol of this study must be communicated to the Study Director as soon as possible. The Study Director must sign a deviation statement including the reason for the deviation and the effect on the study. A summary listing of the deviations will be appended in the final report.

SAFETY AND HEALTH

All laboratory personnel have been trained according to OSHA regulations and practice these guidelines throughout the course of the experimentation. The Sponsor, however, must provide all pertinent Material Safety Data Sheets (MSDS) for the test substance and all active ingredients in the study. The MSDS will be available to all personnel involved in the study.

AWA COMPLIANCE

This study plan has been reviewed and approved by the IACUC of Genesis Laboratories, Inc. in compliance with the Animal Welfare Act. Copies of the approved protocol, compliance documentation provided by the Study Director, and committee review records are on file with the IACUC.

FINAL REPORT

A draft report will be prepared by the Study Director at the conclusion of the study. After receipt of the Sponsor's comments, a final report will be issued. The report will include, but not necessarily be limited to, the following:

A. Name and address of the facility performing the study and the dates on which the study was initiated, completed, terminated, or discontinued.

B. Objectives and procedures stated in the approved protocol, including any changes to the original protocol.

C. Statistical methods employed for analyzing the data.

D. The test device identified by name and any other descriptive terms or numbers.

E. Stability, and, when relevant to the conduct of the study the solubility of the test, control, and reference substances under the conditions of administration.

F. A description of the methods used.

G. A description of the test system used. Where applicable, the final report shall include the number of animals used, sex, body weight range, source of supply, species, strain and sub-strain, age, and procedure used for identification.

H. A description of the dosage, dosage regimen, route of administration, and duration.

I. A description of all circumstances that may have affected the quality or integrity of the data.

J. The name of the Study Director, the names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

K. A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis.

L. The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request of the testing facility or Sponsor, conducted an analysis or evaluation of data after data generation was completed.

M. The locations where all specimens, raw data, and the final report are to be stored.

N. A confidentiality statement worded by and to be signed by the Sponsor.

O. Copies of all raw data sheets.

PROTOCOL AMENDMENT #1

1. **STUDY NUMBER** N05007

2. **STUDY TITLE** Performance Evaluation of Fresh Cab Mouse Repellant for Storage Areas on Wild House Mice (*Mus musculus*)

3. **SPONSOR** Crane Creek Gardens

4. **AMENDMENT TO THE PROTOCOL:** List section(s) affected: **Test System**

Observations were made as to the interactions of the 5 mice within each treatment and control group pairing. It was thought the dominant individuals may be forcing other mice into non-preferred areas. We decided to test only 1 mouse within each treatment/control group pairing. The study may be increased in duration to accommodate this change in the middle of the study.

5. **REASON FOR THE AMENDMENT:**

By using only 1 mouse per treatment/control group pairing, no intraspecies interactions would be possible.

6. **EFFECT OF THE AMENDMENT ON THE STUDY:**

The amendment is being conducted to limit possible confounding factors. This will only improve the integrity of the study.

7. **AMENDMENT APPROVAL**

Jeff A. Mach — Study Director — Date: 5/10/05

Genesis Management — Date

Kari A. Warberg — Sponsor/Sponsor Representative — Date: 5/9/05

IACUC Representative (if required) — Date